02-17-10

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	: Ning Hu et al.	Art Unit	: 1612
Serial No.	: 10/723,431	Examiner	: G.S. Kishore
Filed	: November 26, 2003	Docket	: 01992.007US1
Title	: METHOD OF DRUG LOADING IN LIPOSOMES BY GRADIENT		

APPEAL BRIEF

Mail Stop Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

A final Office Action for the above-identified patent application was mailed January 12, 2009. A Notice of Appeal was filed electronically and received at the United States Patent and Trademark Office on July 13, 2009.

Applicant petitions for a five month extension of time, thereby extending the time-period for submitting the Appeal Brief from September 13, 2009 to February 13, 2010. Please charge the extension fee due to Deposit Account No. 50-3503.

CERTIFICATE OF TRANSMISSION

I hereby certify that this correspondence is transmitted by facsimile, by electronic transmission, or is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

February 16, 2010
Date of Deposit

Lynda Mau
Signature

Lynda Mau
Typed or Printed Name of Person Signing Certificate

i) REAL PARTY IN INTEREST

The real party in interest is Gilead Sciences, Inc. The right of Gilead Sciences Inc. to take action in the subject application was established by virtue of an assignment from the inventors to Gilead Sciences Inc. as recorded at Reel 016496, Frame 0788.

ii) RELATED APPEALS AND INTERFERENCES

There are no related appeals or interferences.

iii) STATUS OF CLAIMS

The final Office action mailed on January 12, 2009 rejected claims 1-28, 30, 31, 33, 40-42 and 47-71. No claims have been allowed. Claims 29, 32, 34-39 and 43-46 have been canceled. Therefore, the rejection of claims 1-28, 30, 31, 33, 40-42 and 47-71 is respectfully appealed.

iv) STATUS OF AMENDMENTS

No amendments have been filed subsequent to the final Office action dated January 12, 2009.

v) SUMMARY OF CLAIMED SUBJECT MATTER

The invention provides a method for encapsulation of pharmaceutical agents in liposomes having a high drug:lipid ratio. The invention also includes gradient loaded liposomes prepared by these methods as well as methods to prepare pharmaceutical compositions comprising said liposomes.

Independent Claim 1 provides a method of forming a gradient loaded liposome. Support for claim 1 can be found at page 18, line 1, page 23, line 27 and page 13, line 24.

A method of forming gradient loaded liposomes, the method comprising:

(a) contacting liposomes in an aqueous solution of at least about 60 mM of an acid with a pharmaceutical agent selected from an anthracycline chemotherapeutic agent, an anthracenedione, and a vinca alkaloid, at a temperature wherein the protonated form of the pharmaceutical agent is charged and is not capable of permeating the membrane of the liposomes, and wherein the unprotonated form of the pharmaceutical agent is uncharged and is capable of permeating the membrane of the liposomes;

(b) actively loading the liposomes with the pharmaceutical agent by raising the pH of the solution to 5.0 or above;

(c) cooling the solution to a temperature at which the unprotonated form of the pharmaceutical agent is not capable of permeating the membrane of the liposomes; and

(d) contacting the solution with a weak base that is an ammonium salt or an alkyl amine, in an amount effective to raise the pH of the internal liposome to provide gradient loaded liposomes.

Independent Claim 63 provides a method for preparing a pharmaceutical composition of a gradient loaded liposome. Support for claim 63 can be found at page 26, line 1, page 23, line 27 and page 13, line 24.

A method for preparing a pharmaceutical composition comprising:

(a) contacting liposomes in an aqueous solution of at least about 60 mM of an acid with a pharmaceutical agent selected from an anthracycline chemotherapeutic agent,

an anthracenedione, and a vinca alkaloid, at a temperature wherein the protonated form of the pharmaceutical agent is charged and is not capable of permeating the membrane of the liposomes, and wherein the unprotonated form of the pharmaceutical agent is uncharged and is capable of permeating the membrane of the liposomes;

(b) actively loading the liposomes with the pharmaceutical agent by raising the pH of the solution to 5.0 or above;

(c) cooling the solution to a temperature at which the unprotonated form of the pharmaceutical agent is not capable of permeating the membrane of the liposomes;

(d) contacting the solution with a weak base that is an ammonium salt or an alkyl amine, in an amount effective to raise the pH of the internal liposome to provide gradient loaded liposomes; and

(e) combining the gradient loaded liposomes with a pharmaceutically acceptable carrier to provide the pharmaceutical composition.

Independent Claim 71 provides a gradient loaded liposome prepared by a process of the invention. Support for claim 71 can be found at page 26, line 17, page 23, line 27 and page 13, line 24.

A gradient loaded liposome prepared by the process comprising:

(a) contacting liposomes in an aqueous solution of at least about 60 mM of an acid with a pharmaceutical agent selected from an anthracycline chemotherapeutic agent, an anthracenedione, and a vinca alkaloid, at a temperature wherein the protonated form of the pharmaceutical agent is charged and is not capable of permeating the membrane of the liposomes, and wherein the unprotonated form of the pharmaceutical agent is uncharged and is capable of permeating the membrane of the liposomes;

(b) actively loading the liposomes with the pharmaceutical agent by raising the pH of the solution to 5.0 or above;

(c) cooling the solution to a temperature at which the unprotonated form of the pharmaceutical agent is not capable of permeating the membrane of the liposomes; and

(d) contacting the solution with a weak base that is an ammonium salt or an alkyl amine, in an amount effective to raise the pH of the internal liposome to provide gradient loaded liposomes.

vi) GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

(1) Whether claims 1-28, 30-31, 33, 40-42 and 47-71 are unpatentable under 35 U.S.C. 103(a) over WO 99/13816 in combination with Tardi (US 2003/0124181).

(2) Whether claims 7 and 49 are unpatentable under 35 U.S.C. 103(a) over WO 99/13816 in combination with Tardi, further in view of Webb (5,814,335).

(3) Whether claims 52-57 are unpatentable under 35 U.S.C. 103(a) over WO 99/13816 in combination with Tardi (US 2003/0124181), further in view of Clerc (5,939,096).

(4) Whether claims 1-28, 30-31, 33, 40-42 and 47-71 are unpatentable on the grounds of nonstatutory obviousness-type double patenting over claims 30-31 and 35-64 of U.S. Patent No. 6,740,335 in combination with Tardi (US 2003/0124181).

vii) ARGUMENT

Rejections Under 35 U.S.C. § 103(a)

The Supreme Court set out the analysis for patentability under 35 USC 103(a): the scope and content of the prior art are to be determined; differences between the prior art and the claims at issue are to be ascertained; and the level of ordinary skill in the pertinent art resolved. Against this background the obviousness or nonobviousness of the subject matter is determined (*see*, *Graham v. John Deere Co.*, 383 U.S. 1 (1966)). The Supreme Court has explained that the Federal Circuit's "teaching, suggestion or motivation) test provides helpful insight into the obviousness question as long as it is not applied rigidly (see, *KSR International Co. v. Teleflex Inc.*, 127 S. Ct. 1727 (2007)).

The Examiner erred in rejecting claims 1-28, 30-31, 33, 40-42 and 47-71 under 35 U.S.C. 103(a) as being unpatentable over WO 99/13816 in combination with Tardi (US 2003/0124181).

Claim 1

Independent claim 1 recites the following:

> A method of forming gradient loaded liposomes, the method comprising:
>
> (a) contacting liposomes in an aqueous solution of at least about 60 mM of an acid with a pharmaceutical agent selected from an anthracycline chemotherapeutic agent, an anthracenedione, and a vinca alkaloid, at a temperature wherein the protonated form of the pharmaceutical agent is charged and is not capable of permeating the membrane of the liposomes, and wherein the unprotonated form of the pharmaceutical agent is uncharged and is capable of permeating the membrane of the liposomes;
>
> (b) actively loading the liposomes with the pharmaceutical agent by raising the pH of the solution to 5.0 or above;
>
> (c) cooling the solution to a temperature at which the unprotonated form of the pharmaceutical agent is not capable of permeating the membrane of the liposomes;

(d) contacting the solution with a weak base that is an ammonium salt or an alkyl amine, in an amount effective to raise the pH of the internal liposome to provide gradient loaded liposomes.

WO 99/13816 discusses a method for loading liposomes with camptothecins. At page 2 of the final Office action mailed January 12, 2009, the Examiner notes that what is lacking in WO is the loading of active agents other than camptothecins. At page 3 of the same final Office action, the Examiner states that Tardi teaches that therapeutic agents that comprise one or more ionizable moiety can be loaded using pH gradients. It is noted that Tardi primarily focuses on the preparation of liposomes from negatively charged lipids that are stable in the blood (please see Tardi at the Abstract, and at paragraphs 0013-0020). At paragraphs 0072-0083, Tardi does generally discuss both the passive and active loading of liposomes. However, gradient loading is not the focal point of the Tardi invention.

The Examiner has taken the position that it would have been obvious from the teaching of Tardi to load other agents such as anthracyclines and vinca alkaloids recited in the instant claims using the method discussed in WO 99/13816.

The instant claims include a cooling (c) and a quenching step (d), wherein the quenching step recites contacting the solution with a weak base that is an ammonium salt or an alkyl amine, in an amount effective to raise the pH of the internal liposome to provide gradient loaded liposomes. As discussed at page 14 of the specification,

Drug loading via the pH gradient includes a low pH in the internal aqueous space of the liposomes, and this internal acidity is, by design, incompletely neutralized during the drug loading process. This residual internal acidity can cause chemical instability in the liposomal preparation (e.g., lipid hydrolysis), leading to limitations in shelf life. To quench this residual internal acidity, membrane permeable bases, such as amines (e.g., ammonium salts or alkyl-amines) can be added following the loading of the pharmaceutical agent in an amount sufficient to reduce the residual internal acidity to a minimum value (for example, pH at or above 4).

The cooling and quenching steps represent significant differences between the teaching of Tardi and the instant claims.

In particular, Tardi does not teach the preparation of any liposomes using the quenching step (d). Accordingly, the final liposomes prepared by Tardi maintain a low pH in the internal aqueous space that helps keep the drug loaded inside the liposome. At paragraph 0076 Tardi teaches that "Once the drug moves inside the liposome, the pH of the interior results in a charged drug state, which prevents the drug from permeating the lipid bilayer, thereby entrapping the drug in the liposome." Additionally in paragraph 0080, Tardi teaches that "Conversion of moiety to a charged form causes the drug to remain encapsulated within the liposome." Accordingly, Tardi teaches that it is critical to maintain the low pH in the internal aqueous space after active loading of the liposome in order to keep the therapeutic agent trapped inside.

Applicant respectfully submits that one skilled in the art would not have had a reasonable belief that the recited anthracyclines and the vinca alkaloids generally discussed in Tardi could have been loaded using the method of WO 99/13816 to provide a loaded liposome that would retain the recited anthracycline or the vinca alkaloid following the quenching procedure of WO 99/13816, since Tardi teaches that the low pH in the interior of the liposome is critical for keeping the agent inside the liposome following gradient loading. Thus, Tardi teaches away from employing the quenching step (d) recited in the instant claims. Accordingly, it is respectfully submitted that one skilled in the art would not have been motivated to combine the teaching of WO 99/13816 and Tardi as suggested by the Examiner. It is respectfully reitterated that one skilled in the art would not have had a reasonable expectation that the liposomes discussed by Tardi would have effectively retained the recited anthracycline chemotherapeutic agent, anthracenedione or the vinca alkaloid if the residual internal acidity was quenched following loading using the method of WO 99/13816.

Additionally, at page 2, of the final Office action mailed January 12, 2009, the Examiner states that "Although in Examples, WO uses citric acid at 50 mM concentration, in view of WO's teachings that it can be higher than 5 mM, it would have been obvious to one of ordinary skill in the art to vary the molality with the expectation of obtaining the best possible results." Applicant respectfully disagrees

with the Examiner's interpretation of WO 99/13816.

One skilled in the art would have believed that gradient loading using a high concentration of citric acid would produce a final liposome with a higher concentration of therapeutic agent in the liposome than would be produced by gradient loading at a lower concentration of citric acid. Accordingly one skilled in the art would have known that gradient loading using a higher concentration of acid would produce a final liposome that is potentially less thermodynamically stable, i.e. a liposome wherein the therapeutic agent is more likely to leak or escape from the liposome. As discussed above, Tardi teaches that it is critical to maintain low pH in the internal aqueous space after active loading of the liposome in order to keep the therapeutic agent trapped inside. One skilled in the art would have understood that the teaching of Tardi would have been more relevant for liposomes loaded at higher gradients, since these liposomes contain more therapeutic agent and thus, are more likely to leak if the pH gradient is quenched. For this additional reason, it is submitted that it would not have been obvious to one of ordinary skill in the art to vary the molality (by increasing the concentration of citric acid) with the expectation of obtaining the best possible results, as suggested by the Examiner. In fact, it is submitted that one skilled in the art would have more likely assumed that quenching liposomes that were gradient loaded at a high concentration of citric acid would have produced liposomes that were unstable (i.e. liposomes where the therapeutic agent would leak from the liposome) in light of the discussion in Tardi.

Since there was no motivation to combine the cited documents as suggested by the Examiner, and because there would not have been a reasonable expectation that the references so combined would have provided liposomes that would have sufficiently retained the therapeutic agent, it is respectfully submitted that the Office has not established a *prima facie* case of obviousness over WO 99/13816 in combination with Tardi.

Regarding WO99/13816, it is stated at page 12, lines 22-23 of WO99/13816 "The preferred buffers are >5 mM, more preferably 50 mM..." This statement is made even though WO99/13816 describes the loading and quenching of a liposome, albeit for a stability study, using an acid concentration of 100 mM as described at page 22, lines

10-15. Given this preference of buffer concentration of 50 mM in light of a study with a higher buffer concentration, WO99/13816 effectively teaches away from the acid concentration of at least about 60 mM or higher as recited in the instant claims. Therefore, one skilled in the art would not have been motivated to use an acid concentration of at least about 60 mM to load liposomes, as recited in the instant claims, with a reasonable expectation of success.

For these reasons Applicant requests that the Board reverse the Examiner's rejection of claim 1 as being obvious over WO 99/13816 in combination with Tardi.

Claims 2-6, 8-28, 30, 31, 33, 40, 41, 47, 48 and 50-61

Claims 2-6, 8-28, 30, 31, 33, 40, 41, 47, 48 and 50-61 are directly or indirectly dependent on claim 1. For reasons analogous to those stated above, the Office fails to establish a *prima facie* case of obviousness with respect to any of claims 2-6, 8-28, 30, 31, 33, 40, 41, 47, 48 and 50-61. Therefore, Applicant requests that the Board reverse the Examiner's rejection of claims 2-6, 8-28, 30, 31, 33, 40, 41, 47, 48 and 50-61 as being obvious over WO 99/13816 in combination with Tardi.

Claim 7

Claim 7 is dependent on claim 1 and recites "wherein the non-phosphatidyl lipids comprise sphingomyelin." At page 3 and again at page 4 of the Office action mailed May 14, 2008, the Examiner admitted that WO 99/13816 in combination with Tardi do not teach sphingomyelin as a liposome-forming lipid. Accordingly, the cited documents do not teach all elements of claim 7. Thus, claim 7 cannot be *prima facie* obvious over the cited documents. Additionally, at page 3 of the Office action mailed May 14, 2008 the Examiner states that "since it is a commonly used lipid in the liposome formations, it would have been obvious to one of ordinary skill in the art to use this lipid with a reasonable expectation of success." This line of reasoning is further exemplified at page 5 of the final Office action mailed January 12, 2009, which concludes that the Applicant's arguments are not persuasive since sphingomyelin is known in the art as a liposome forming lipid. It is respectfully submitted that the Examiner has provided no evidence to support this line of reasoning. Thus, the Examiner has not met his burden

and the rejection is inappropriate. In the response filed November 14, 2008 Applicant noted that the Examiner's failure to provide any supporting evidence means that the Examiner is taking "official notice" that "it would have been obvious to one of ordinary skill in the art to use this lipid with a reasonable expectation of success." The Office has not provided an affidavit or declaration setting forth specific factual statements and explanations to support this finding as required under 37 C.F.R. 1.104(d)(2). For these reasons and those stated above, the Office fails to establish a *prima facie* case of obviousness with respect to claims 7. Accordingly, Applicant requests that the Board reverse the Examiner's rejection of claim 7 as being obvious over WO 99/13816 in combination with Tardi.

Claim 42

Claim 42 is dependent on claim 1 and recites "...wherein the solution is cooled in step (c) to a temperature of about 0 °C to about 30 °C." For reasons analogous to those stated above, the Office fails to establish a *prima facie* case of obviousness with respect to claim 42. In addition, claim 42 is independently patentable as the primary reference (WO 99/13816) does not teach cooling the solution of liposomes to a temperature of about 0 °C to about 30 °C (step c) prior to contacting the solution with a weak base. Please note as mentioned above that Tardi does not teach steps (c) or (d) so does not remedy the deficiencies of WO 99/13816 with respect to claim 42. Document WO99/13816 does discuss cooling the temperature of the liposomal solution from 55 °C to below 35 °C prior to contacting the solution with ammonium chloride (page 21, line 23). However, WO99/13816 is silent regarding the purpose of cooling the solution. In contrast, instant claim 1 clearly states that the purpose of cooling the liposomal solution of step (c) is to prevent the unprotonated form of the pharmaceutical agent from permeating the membrane of the microsomes. Therefore, WO 99/13816 does not teach or provide any motivation to utilize a lower temperature range (about 0 °C to about 30 °C) when cooling the liposomes prior to base treatment, as described in the instant claims. For this additional reason, the Office fails to establish a *prima facie* case of obviousness with respect to claim 42. Accordingly, Applicant requests that the Board reverse the Examiner's rejection of claim 42 as being obvious over WO 99/13816 in

combination with Tardi.

Claim 49

Claim 49 is dependent on claim 1 and recites "...wherein the base is alkyl-amine selected from the group of methylamine, ethyl amine....." For reasons analogous to those stated above, the Office fails to establish a *prima facie* case of obviousness with respect to claim 49. In addition, at page 4 of the Office action mailed May 14, 2008, the Examiner admitted that WO99/13816 does not teach the change of the pH using methylamine. Accordingly, WO99/13816 and Tardi do not teach all elements of claim 49. Thus, claim 49 cannot be *prima facie* obvious over the cited documents. At page 5 of the final Office action the Examiner states that the prior art clearly indicates that methylamine is a commonly used base in liposome technology. However, the Examiner has provided no evidence or line of reasoning to substantiate this statement and has thus taken "official notice" with no accompanying declaration or affidavit as required under 37 C.F.R. 1.104(d)(2). For this additional reason, the Office fails to establish a *prima facie* case of obviousness with respect to claim 49. Accordingly, Applicant requests that the Board reverse the Examiner's rejection of claim 39 as being obvious over WO 99/13816 in combination with Tardi.

Claim 63

Independent claim 63 recites the following:

> A method for preparing a pharmaceutical composition comprising:
>
> (a) contacting liposomes in an aqueous solution of at least about 60 mM of an acid with a pharmaceutical agent selected from an anthracycline chemotherapeutic agent, an anthracenedione, and a vinca alkaloid, at a temperature wherein the protonated form of the pharmaceutical agent is charged and is not capable of permeating the membrane of the liposomes, and wherein the unprotonated form of the pharmaceutical agent is uncharged and is capable of permeating the membrane of the liposomes;

(b) actively loading the liposomes with the pharmaceutical agent by raising the pH of the solution to 5.0 or above;

(c) cooling the solution to a temperature at which the unprotonated form of the pharmaceutical agent is not capable of permeating the membrane of the liposomes;

(d) contacting the solution with a weak base that is an ammonium salt or an alkyl amine, in an amount effective to raise the pH of the internal liposome to provide gradient loaded liposomes.

(e) combining the gradient loaded liposomes with a pharmaceutically acceptable carrier to provide the pharmaceutical composition.

Claim 63 recites a method for preparing a pharmaceutical composition which comprises the method of forming gradient loaded liposomes as recited in claim 1 and further combining the gradient loaded liposomes with a pharmaceutically acceptable carrier. For reasons analogous to those provided in support of the patentability of claim 1 (pages 11-14 of this paper), Applicant asserts that the Office fails to establish a *prima facie* case of obviousness with respect to claim 63. Therefore, Applicant requests that the Board reverse the Examiner's rejection of claim 63 as being obvious over WO 99/13816 in combination with Tardi.

Claims 64-70

Claims 64-70 are dependent either directly or indirectly on claim 63. For reasons analogous to those stated above, the Office fails to establish a *prima facie* case of obviousness with respect to any of claims 64-70. Therefore, Applicant requests that the Board reverse the Examiner's rejection of claims 64-70 as being obvious over WO 99/13816 in combination with Tardi.

Claim 71

Independent claim 71 recites the following:

A gradient loaded liposome prepared by the process comprising:

(a) contacting liposomes in an aqueous solution of at least about 60 mM of an acid with a

pharmaceutical agent selected from an anthracycline chemotherapeutic agent, an anthracenedione, and a vinca alkaloid, at a temperature wherein the protonated form of the pharmaceutical agent is charged and is not capable of permeating the membrane of the liposomes, and wherein the unprotonated form of the pharmaceutical agent is uncharged and is capable of permeating the membrane of the liposomes;

(b) actively loading the liposomes with the pharmaceutical agent by raising the pH of the solution to 5.0 or above;

(c) cooling the solution to a temperature at which the unprotonated form of the pharmaceutical agent is not capable of permeating the membrane of the liposomes; and

(d) contacting the solution with a weak base that is an ammonium salt or an alkyl amine, in an amount effective to raise the pH of the internal liposome to provide gradient loaded liposomes.

Claim 71 recites a gradient loaded liposome prepared by the method recited in claim 1. For reasons analogous to those provided in support of the patentability of claim 1 (pages 11-14 of this paper), Applicant asserts that the Office fails to establish a *prima facie* case of obviousness with respect to claim 71. Therefore, Applicant requests that the Board reverse the Examiner's rejection of claim 71 as being obvious over WO 99/13816 in combination with Tardi.

The Examiner erred in rejecting claims 7 and 49 under 35 U.S.C. 103(a) as being unpatentable over WO 99/13816 in combination with Tardi, further in view of Webb (5,814,335).

Claim 7

Claim 7 is dependent on claim 1 and recites "wherein the non-phosphatidyl lipids comprise sphingomyelin." For reasons stated above, the Office fails to establish a *prima facie* case of obviousness over primary reference WO99/13816 in combination with Tardi. Webb (5,814,335) was cited with respect to sphingomyelin as a liposome forming lipid. The Examiner has not provided any discussion suggesting how Webb remedies any of the deficiencies in the primary reference and Tardi. Accordingly, the Office has

failed to establish a *prima facie* case of obviousness with respect to claim 7. Therefore, Applicant requests that the Board reverse the Examiner's rejection of claim 7 as being obvious over WO 99/13816 in combination with Tardi in view of Webb.

Claim 49

Claim 49 is dependent on claim 1 and recites "...wherein the base is alkyl-amine selected from the group of methylamine, ethyl amine....." For reasons stated above, the Office fails to establish a *prima facie* case of obviousness over primary reference WO99/13816 in combination with Tardi. Webb (5,814,335) was also cited with respect to the use of methylamine. The Examiner has not provided any discussion suggesting how Webb remedies any of the deficiencies in the primary reference and Tardi.

In addition to the fact that the rejection should be withdrawn for the reason discussed in the paragraph above, it is respectfully submitted that methyl amine/methyl ammonium gradient discussed in Webb performs a significantly different function than the methyl amine recited in claim 49. Webb discusses the use of a methyl amine/methyl ammonium gradient to actively load the neutral form of a protonatable therapeutic agent (column 7, lines 40-64) into a liposome. Claim 49 is directed to the method of claim 1 wherein a weak base selected from the group of methyl amine, ethyl amine, diethyl amine, ethylene diamine, and propyl amine is used in step (d) to quench the residual acidity inside the liposomes after loading in the presence of an acid.

Webb does not use the methylamine to quench residual acid in a loaded liposome. Rather, Webb uses methylamine to establish a pH gradient (as noted by the Examiner at page 5 of the Office action mailed May 14, 2008). Thus, methyl amine is used by Webb for the opposite purpose than it is used for in the claimed methods. Accordingly, it is submitted that one skilled in the art would not have found any motivation in Webb to use methylamine as recited in claim 49. For these reasons it is submitted that the Office fails to establish a *prima facie* case of obviousness with respect to claim 49. Accordingly, Applicant requests that the Board reverse the Examiner's rejection of claim 49 as being obvious over WO 99/13816 in combination with Tardi in view of Webb.

The Examiner erred in rejecting claims 52-57 under 35 U.S.C. 103(a) as being unpatentable over WO 99/13816 in combination with Tardi (US 2003/0124181), further in view of Clerc (5,939,096).

Claims 52-57

Claims 52-57 are dependent either directly or indirectly on claim 1 and relate to the dehydration of the liposomes of claim 1 as well as the rehydration of said dehydrated liposomes. For reasons stated above, the Office fails to establish a *prima facie* case of obviousness over primary reference WO99/13816 in combination with Tardi. Clerc (5,939,096) was only cited with respect to the dehydration of liposomes in the presence of cryoprotectants. The Examiner has not provided any discussion suggesting how Clerc remedies any of the deficiencies in the primary reference and Tardi. Accordingly, the Office has failed to establish a *prima facie* case of obviousness with respect to claims 52-57. Therefore, Applicant requests that the Board reverse the Examiner's rejection of claims 52-57 as being obvious over WO99/13816 in combination with Tardi in view of Clerc.

The Examiner erred in rejecting claims 1-28, 30-31, 33, 40-42 and 47-71 on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 30-31 and 35-64 of U.S. Patent No. 6,740,335 in combination with Tardi (US 2003/0124181).

Claims 1-28, 30-31, 33, 40-42 and 47-71

WO 99/13816 (discussed above) is a foreign counterpart for U.S. Patent 6,740,335. Claims 31-30 and 35-64 of U.S. Patent Number 6,740,335 discuss processes of preparing liposomes with a camptothecin dereivative. However, none of the cited claims of U.S. Patent 6,740,335 recite certain elements of the independent claims (1, 63, 71) of the instant application including "contacting liposomes in an aqueous solution of at least about 60 mM of an acid with a pharmaceutical agent" or "cooling the solution to a temperature at which the unprotonated form of the pharmaceutical agent is not capable of permeating the membrane of the liposomes." In addition, for reasons

analogous to those stated on pages 11-14 of this paper, Applicant notes that there is no motivation to combine Tardi and the claims of U.S. Patent 6,740,335 (WO 99/13816) as suggested by the Examiner to arrive at the claims of the instant application, and in particular, the recited pharmaceutical agents (anthracycline chemotherapeutic agent, an anthracenedione, and a vinca alkaloid). Therefore, the Office fails to establish a *prima facie* case of nonstatutory obviousness-type double patenting with respect to any of claims 1-28, 30-31, 33, 40-42 and 47-71.

Regarding the concentration of the acid recited in the instant claims (i.e. 60 mM), Applicant notes that, at page 8 of the final Office action mailed January 12, 2009, the Examiner appears to remedy the lack of the recited acid concentration element by stating, "Patented claims do not recite the concentration of the acid while loading the active agent and instant mM amounts therefore, are deemed to be anticipated by the claims of the patent." It is not clear why the Examiner is discussing anticipation in the remarks to the obviousness-type double patenting rejection of the instant claims. It is also respectfully submitted that the Examiner's statement is contrary to well established law regarding anticipation.

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration. *In re Dillon*, 919 F.2d 688, 16 U.S.P.Q.2d 1897, 1908 (Fed. Cir. 1990) (en banc), cert. denied, 500 U.S. 904 (1991). For anticipation, there must be no difference between the claimed invention and the reference disclosure, as viewed by a person of ordinary skill in the art. *Scripps Clinic & Res. Found. v. Genentech, Inc.*, 927 F.2d 1565, 18 USPQ2d 101 (Fed. Cir. 1991).

The instant claims are directed to a method of forming gradient loaded liposomes, the method comprising: (a) contacting liposomes in an aqueous solution of at least about 60 mM of an acid.... The claims specifically recite "an aqueous solution of at least about 60 mM of an acid." This element is not found in the claims of U.S. Patent Number 6,740,335. Accordingly, the Examiner's statement regarding the anticipatory effect of the cited claims is incorrect.

For these reasons the Office again fails to establish a prima facie case of nonstatutory obviousness-type double patenting with respect to any of claims 1-28, 30-31, 33, 40-42 and 47-71. Accordingly, Applicant requests that the Board reverse the

Examiner's rejection of claims 1-28, 30-31, 33, 40-42 and 47-71 on the ground of nonstatutory obviousness-type double patenting over U.S. Patent No. 6,740,335 in combination with Tardi

Applicant respectfully submits that the claims are in condition for allowance, and notification to that effect is respectfully requested. If necessary, please charge any additional fees or credit overpayment to Deposit Account 50-3503.

Respectfully submitted,
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viii) CLAIMS APPENDIX

1. A method of forming gradient loaded liposomes, the method comprising:

(a) contacting liposomes in an aqueous solution of at least about 60 mM of an acid with a pharmaceutical agent selected from an anthracycline chemotherapeutic agent, an anthracenedione, and a vinca alkaloid, at a temperature wherein the protonated form of the pharmaceutical agent is charged and is not capable of permeating the membrane of the liposomes, and wherein the unprotonated form of the pharmaceutical agent is uncharged and is capable of permeating the membrane of the liposomes;

(b) actively loading the liposomes with the pharmaceutical agent by raising the pH of the solution to 5.0 or above;

(c) cooling the solution to a temperature at which the unprotonated form of the pharmaceutical agent is not capable of permeating the membrane of the liposomes; and

(d) contacting the solution with a weak base that is an ammonium salt or an alkyl amine, in an amount effective to raise the pH of the internal liposome to provide gradient loaded liposomes.

2. The method of claim 1 wherein the liposomes comprise phosphatidylcholine.

3. The method of claim 1 wherein the liposomes comprise phosphatidylcholine selected from the group of distearoylphosphatidylcholine, hydrogenated soy phosphatidylcholine, hydrogenated egg phosphatidylcholine, dipalmitoylphosphatidylcholine, dimyristoylphosphatidylcholine, and dielaidoyl phosphatidyl choline.

4. The method of claim 1 wherein the liposomes further comprise cholesterol.

5. The method of claim 1 wherein the liposomes further comprise phosphatidylglycerol.

6. The method of claim 1 wherein the liposomes further comprise non-phosphatidyl lipids.

7. The method of claim 6 wherein the non-phosphatidyl lipids comprise sphingomyelin.

8. The method of claim 1 wherein the liposomes further comprise phosphatidylglycerol selected from the group of dimyristoylphosphatidylglycerol, dilaurylphosphatidylglycerol, dipalmitoylphosphatidylglycerol, and distearoylphosphatidylglycerol.

9. The method of claim 1 wherein the liposomes comprises phosphatidylcholine, and further comprises cholesterol.

10. The method of claim 1 wherein the liposomes comprises phosphatidylcholine, and further comprises cholesterol, wherein the molar ratio of the phosphatidylcholine to the cholesterol is about 1:0.01 to about 1:1.

11. The method of claim 1 wherein the liposomes comprises phosphatidylcholine, and further comprises cholesterol, wherein the molar ratio of the phosphatidylcholine to the cholesterol is about 1.5:1.0 to about 3.0:1.0.

12. The method of claim 1 wherein the liposomes are unilamellar and less than about 100nm.

13. The method of claim 1 wherein the weight ratio of the liposomes to the pharmaceutical agent is up to about 200:1.

14. The method of claim 1 wherein the weight ratio of the liposomes to the pharmaceutical agent is about 1:1 to about 100:1.

15. The method of claim 1 wherein the weight ratio of the liposomes to the pharmaceutical agent is about 1:1 to about 50:1.

16. The method of claim 1 wherein the acid has an acid dissociation constant of less than about 1×10^{-2}.

17. The method of claim 1 wherein the acid has an acid dissociation constant of less than about 1×10^{-4}.

18. The method of claim 1 wherein the acid has an acid dissociation constant of less than about 1×10^{-5}.

19. The method of claim 1 wherein the acid has a permeability coefficient larger than about 1×10^{-4} cm/sec for the liposomes.

20. The method of claim 1 wherein the acid is selected from the group of formic acid, acetic acid, propanoic acid, butanoic acid, pentanoic acid, citric acid, oxalic acid, succinic acid, lactic acid, malic acid, tartaric acid, fumaric acid, benzoic acid, aconitic acid, veratric acid, phosphoric acid, sulfuric acid, and combinations thereof.

21. The method of claim 1 wherein the acid is citric acid.

22. The method in claim 1 wherein at least about 100 mM of an acid is employed.

23. The method of claim 1 wherein the pharmaceutical agent exists in a charged state when dissolved in an aqueous medium.

24. The method of claim 1 wherein the pharmaceutical agent is an organic compound that includes at least one acyclic or cyclic amino group, capable of being protonated.

25. The method of claim 1 wherein the pharmaceutical agent is an organic compound that includes at least one primary amine group, at least one secondary amine group, at

least one tertiary amine group, at least one quaternary amine group, or any combination thereof.

26. The method of claim 1 wherein the pharmaceutical agent is an antineoplastic agent.

27. The method of claim 1 wherein the pharmaceutical agent is a combination of two or more antineoplastic agents.

28. The method of claim 1 wherein the pharmaceutical agent is an ionizable basic antineoplastic agent.

30. The method of claim 1 wherein the anthracycline chemotherapeutic agent is selected from the group of doxorubicin, epirubicin, and daunorubicin.

31. The method of claim 1 wherein the anthracenedione is mitoxantrone.

33. The method of claim 1 wherein the vinca alkaloid is selected from the group of vincristine and vinblastine.

40. The method of claim 1 wherein the temperature in step (a) is about 40°C to about 70°C.

41. The method of claim 1 wherein the temperature in step (a) is about 50°C to about 60°C.

42. The method of claim 1 wherein the solution is cooled in step (c) to a temperature of about 0°C to about 30°C.

47. The method of claim 1 wherein the weak base is an ammonium salt having a mono- or multi-valent counterion.

48. The method of claim 1 wherein the weak base is selected from the group of ammonium sulfate, ammonium hydroxide, ammonium acetate, ammonium chloride, ammonium phosphate, ammonium citrate, ammonium succinate, ammonium lactobionate, ammonium carbonate, ammonium tartarate, ammonium oxalate, and combinations thereof.

49. The method of claim 1 wherein the weak base is alkyl-amine selected from the group of methyl amine, ethyl amine, diethyl amine, ethylene diamine, and propyl amine.

50. The method of claim 1 further comprising, during or after step (d), removing any unloaded pharmaceutical agent.

51. The method of claim 50 wherein the removing of the unloaded drug employs removing the unloaded drug via cross filtration or dialysis.

52. The method of claim 1 further comprising, after step (d), dehydrating the liposomes.

53. The method of claim 52 wherein the dehydrating is carried out at a pressure of below about 1 atm.

54. The method of claim 52 wherein the dehydrating is carried out with prior freezing of the liposomes.

55. The method of claim 52 wherein the dehydrating is carried out in the presence of one or more protective monosaccharide sugars, one or more protective disaccharide sugars, or a combination thereof.

56. The method of claim 55 wherein the protective sugar is selected from the group of trehalose, sucrose, maltose, and lactose.

57. The method of claim 52 further comprising rehydrating the liposomes after the dehydrating.

58. The method of claim 1 wherein the liposomes are unilamellar vescicles.

59. The method of claim 1 wherein the liposomes are multilamellar vescicles.

60. The method of claim 1 wherein more than about 90 wt.% of the pharmaceutical agent is trapped in the liposomes.

61. The method of claim 1 further comprising, after step (d), contacting the liposomes with a pharmaceutically acceptable carrier.

62. The method of claim 1 wherein the acid is present in at least about 200 mM.

63. A method for preparing a pharmaceutical composition comprising:

(a) contacting liposomes in an aqueous solution of at least about 60 mM of an acid with a pharmaceutical agent selected from an anthracycline chemotherapeutic agent, an anthracenedione, and a vinca alkaloid, at a temperature wherein the protonated form of the pharmaceutical agent is charged and is not capable of permeating the membrane of the liposomes, and wherein the unprotonated form of the pharmaceutical agent is uncharged and is capable of permeating the membrane of the liposomes;

(b) actively loading the liposomes with the pharmaceutical agent by raising the pH of the solution to 5.0 or above;

(c) cooling the solution to a temperature at which the unprotonated form of the pharmaceutical agent is not capable of permeating the membrane of the liposomes;

(d) contacting the solution with a weak base that is an ammonium salt or an alkyl amine, in an amount effective to raise the pH of the internal liposome to provide gradient loaded liposomes; and

(e) combining the gradient loaded liposomes with a pharmaceutically acceptable carrier to provide the pharmaceutical composition.

64. A method comprising administering the pharmaceutical composition of claim 63 to a mammal.

65. A method for treating a mammal inflicted with cancer, the method comprising administering the pharmaceutical composition of claim 63 to the mammal, wherein the pharmaceutical agent is an antineoplastic agent.

66. The method of claim 65 wherein the cancer is a tumor, ovarian cancer, small cell lung cancer (SCLC), non small cell lung cancer (NSCLC), leukemia, sarcoma, colorectal cancer, head cancer, neck cancer, or breast cancer.

67. The method of claim 65 wherein the administration of the antineoplastic agent, *via* the liposomal formulation, has a toxicity profile that is lower than the toxicity profile associated with the administration of the antineoplastic agent in the free form.

68. The method of claim 67 wherein the toxicity is selected from the group of gastrointestinal toxicity and cumulative dose-dependent irreversible cardiomyopathy.

69. The method of claim 65 wherein the administration of the antineoplastic agent has unpleasant side-effects that are lower in incidence, severity, or a combination thereof,

than unpleasant side-effects associated with the administration of the antineoplastic agent in the free form.

70. The method of claim 69 wherein the unpleasant side-effects are selected from the group of myelosuppression, alopecia, mucositis, nausea, vomiting, and anorexia.

71. A gradient loaded liposome prepared by the process comprising:

(a) contacting liposomes in an aqueous solution of at least about 60 mM of an acid with a pharmaceutical agent selected from an anthracycline chemotherapeutic agent, an anthracenedione, and a vinca alkaloid, at a temperature wherein the protonated form of the pharmaceutical agent is charged and is not capable of permeating the membrane of the liposomes, and wherein the unprotonated form of the pharmaceutical agent is uncharged and is capable of permeating the membrane of the liposomes;

(b) actively loading the liposomes with the pharmaceutical agent by raising the pH of the solution to 5.0 or above;

(c) cooling the solution to a temperature at which the unprotonated form of the pharmaceutical agent is not capable of permeating the membrane of the liposomes; and

(d) contacting the solution with a weak base that is an ammonium salt or an alkyl amine, in an amount effective to raise the pH of the internal liposome to provide gradient loaded liposomes.

ix) EVIDENCE APPENDIX

A. WO 99/13816

This document was submitted in an Information Disclosure Statement mailed February 9, 2005.

B. US 2003/0124181

This document was entered by the Examiner in the Office Action dated May 14, 2008.

C. US 5,814,335

This document was submitted in an Information Disclosure Statement mailed February 9, 2005.

D. US 5,939,096

This document was entered by the Examiner in the Office Action dated March 27, 2007.

x) RELATED PROCEEDINGS APPENDIX

None.